Role of Endogenous Cytokines Secretion in Radioprotection Conferred by the Immunomodulator Ammonium Trichloro(dioxyethylene-0-0′)tellurate

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The immunomodulator ammonium trichloro(dioxyethylene-0-0′)tellurate (AS101) has previously been found by us to have radioprotective properties when injected into mice before sublethal and lethal doses of irradiation. AS101 also was found to protect mice from hematopoietic damage caused by various chemotherapeutic drugs. Based on these findings, phase II clinical trials with cancer patients treated with AS101, in combination with chemotherapy, are currently underway. In the present study, we wanted to assess the role of several cytokines in the radioprotection conferred by AS101. We show that the administration of neutralizing antibodies against interleukin-1 (IL-1) receptor, IL-6 receptor, IL-6, tumor necrosis factor (TNF), or stem cell factor (SCF) completely abrogates the ability of AS101 to increase the survival of lethally irradiated mice. Moreover, the injection of each of these antibodies reduces the ability of AS101 to increase the number of BM, spleen cells, and the number of circulating neutrophils, lymphocytes, and platelets in irradiated mice. In addition, these antibodies abrogate the enhancing effect of AS101 on the secretion of IL-3, IL-6, and granulocyte-macrophage colony-stimulating factor, all of which decrease significantly in sublethally irradiated mice. By contrast, the injection of anti–IL-2 receptor antibody or control Igs to AS101-treated mice does not interfere with the radioprotective effects of the compound. These results suggest a role for IL-1, IL-6, TNFα, and SCF in the radioprotective effect of AS101. Because cytokine toxicity remains a significant concern, the clinical application of AS101, which has no toxicity, is particularly valuable.

CHEMICAL- OR RADIATION-induced septicemia and death are primarily because of damage of the intestine and hematopoietic system. This treatment invariably leads to a decrease in BM function of variable durations that is reflected in a substantial decrease in mature blood elements.[1,2] In the past few years, various substances that protect the hemopoietic system against adverse effects of cytoreductive treatments have been described. Radioprotectors are either chemical or biologic agents, the administration of which before or during irradiation diminishes radiation-induced damage. The chemical agents are primarily thiol compounds[3] that are known to act by scavenging free radicals. The biologic agents are immunomodulatory and/or inflammatory substances, many of which induce the release of cytokines.[4-6] The most effective and most extensively studied radioprotective cytokines are interleukin-1 (IL-1),[7] tumor necrosis factor α (TNFα),[8] granulocyte colony-stimulating factor (G-CSF),[9] and granulocyte-macrophage CSF (GM-CSF).[10] Recently, treatment with recombinant stem cell factor (rSCF) has also been shown to protect mice from lethal effects of irradiation.[11] SCF has been shown to act on very primitive cell populations that have been described as stem cell populations.[12] The immunomodulator AS101, ammonium trichloro(dioxoethylene-0-0′) tellurate, was developed in Bar Ilan University and previously shown by us to stimulate the production of a variety of cytokines.[13-17] Phase I clinical trials on cancer patients showed an enhancement in the secretion of endogenous TNF, interferon (IFN), and IL-2.[18,19] More recently, AS101 has been shown to have radioprotective properties when injected into mice before sublethal and lethal doses of irradiation.[17,20-21] In addition, AS101 was found to protect mice from hemopoietic damages caused by sublethal doses of cyclophosphamide (CYP) and to increase the rate of survival of mice treated with lethal doses of CYP.[22] Moreover, AS101 was shown to protect bone marrow (BM) progenitor cells and BM stromal cells from toxic effects of the CYP derivative ASTA-Z 7557.[23,24] In all these studies, AS101 prevented damage caused both to BM progenitor cells and to their functions as well as to lymphoid cell reactivity. As a result of these studies, phase II clinical trials with cancer patients treated with AS101 in combination with chemotherapy have been initiated.

In this study, we assessed the relative contribution of various hematopoietic growth factors to the radioprotection conferred by AS101, by using neutralizing antibodies to various cytokines. The results suggest that IL-1, IL-6, TNFα, and the c-*kit* ligand play an important role in the effect of AS101 against lethal and sublethal effects of radiation.

MATERIALS AND METHODS

Mice

Balb/c mice, 2 months of age, were purchased from Jackson Laboratories (Bar Harbor, Maine) and were housed 10 per cage.

Treatment With AS101

AS101 (Ossirene; Baker Norton Pharmaceuticals Inc, Miami, FL) was administered to mice at a concentration of 10 μg/0.2 mL per injection. The compound was supplied in a solution of phosphate-buffered saline (PBS) at pH 7.4 and maintained at 4°C. Before use, AS101 was diluted in PBS, and the appropriate concentration in 0.2-mL volume was administered to mice by intraperitoneal injection. Control animals received 0.2 mL PBS at the same time.

Antibodies

Antibodies used for injection were rat monoclonal IgG_2 antimouse IL-1 receptor (IL-1R; Genzyme, Boston, MA) and the control purified rat IgG_2; rat monoclonal IgG_2 antimouse IL-6R (Biosource, Camarillo, CA). Rat IgG_1 monoclonal antimurine IL-6 was purchased from Biosource. Monoclonal IgG_1 rat antimouse TNF was

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purchased from Pharmingen (San Diego, CA). Control rat IgG_1 Ig (Pharmingen). Monoclonal IgG antimouse IL-2R was purchased from Biodesign (Kennebunk, ME), and the monoclonal rat IgG_2 antimouse SCF was from Genzyme.

Treatment With Antibodies

Antibodies diluted in 0.1 mL PBS were injected into mice simultaneously with AS101 24 hours before irradiation. Control mice were injected with either the respective control Ig or with the antibody alone 24 hours before irradiation. All neutralizing antibodies and their respective control Igs were injected at 100 μg/mouse, except for anti-IL-6 antibodies that were injected at 600 μg/mouse and anti-SCF antibodies that were injected at 50 μg/mouse.

Recovery of BM and Spleen Cells

Femurs and spleens were removed and placed in PBS. Single-cell suspensions of BM were prepared by washing each cavity of the femur with 5 mL PBS with a sterile syringe and a 26-gauge needle. Spleen cells were passed through stainless steel mesh nets, treated with hypotonic solution to lyse erythrocytes, and washed 3 times. Cell counts were obtained using a hemocytometer. Viability, as assessed by trypan-blue exclusion method, was always found to be more than 95%.

Induction of Cytokines Secretion In Vitro

Splenocytes (5×10^6/mL for GM-CSF secretion and 1×10^6/mL for IL-3 and IL-6 secretion) suspended in enriched RPMI-1640 culture medium supplemented with 10% fetal calf serum were seeded in 24-well culture plates in the presence of 2.5 μg/mL concanavalin A (ConA; Biomaker, Rehovot, Israel). The cultures were incubated for 24 hours at 37°C. Supernatants were collected and stored at 4°C.

Quantification of Cytokines

The IL-3-responsive (GM-CSF-unresponsive) cell line 32 DCI-23 was used to assay IL-3 content. 32DCI-23 cells (10^4 per well) were seeded in triplicate in culture medium with or without dilutions of the supernatant fraction. After 48 hours, ^{3}H-thymidine (^{3}H-TdR) uptake was determined in a liquid scintillation counter. One unit of IL-3 activity was defined as the reciprocal log 2 dilution required to give 50% of the maximal proliferation of 10^4 IL-3-dependent 32 DCI-23 cells after 48 hours of culture. IL-6 and GM-CSF were quantified using murine IL-6 or murine GM-CSF enzyme-linked immunoassay kit (Endogen, Boston, MA).

Radiation

Mice were exposed to γ-irradiation and received total irradiation from a cesium-137 radiation source at a dose rate of 450 cGy/min. For survival analysis, mice were irradiated with 750 cGy. For sublethal irradiation, mice were irradiated with 450 cGy.

Statistical Analysis

For multiple group comparisons, the pairwise *t*-test was used. Survival curves were tested both by comparing the cumulative percentage of survival and by the percentage of survival at the termination of the experiment. For the first method, we used the Gehan-Wilcoxon test, and, for the second, we used the χ^2 test for proportions.

RESULTS

The Effect of Cytokine Abrogation on Radioprotection Conferred by AS101

Effect on survival of lethally irradiated mice. We have previously shown that pretreatment of mice with AS101 confers radioprotection in mice by significantly increasing their rate of survival. To determine if the protection afforded by AS101 is caused by enhanced production of specific cytokines, mice were administered with antibodies against either IL-1R, IL-2R, IL-6R, IL-6, TNF, or SCF. Control mice were injected with either saline or the relevant control Igs. Figures 1A through D show that 13 days after irradiation (750 cGy), 100% lethality was observed. Treatment with AS101 24 hours before irradiation increased the rate of survival to 80% to 90%. This increase was significant ($P < .01$) when compared with control PBS-injected mice by either the Gehan-Wilcoxon or the χ^2 test. The results clearly show that pretreatment of mice with either anti-IL-1R, anti-IL-6R, anti-IL-6, anti-TNF, or anti-SCF completely abrogated the radioprotective action of AS101, whereas treatment of control mice with identical concentrations of control Igs or anti-IL-2R antibodies did not affect the rate of survival induced by AS101. No significant differences were observed between the percent of survival of mice treated with AS101 and those treated with AS101 concomitantly with control Igs. The extent of abrogation of the radioprotective effect conferred by AS101 by treatment of the various antibodies was similar (Fig 1A through D). Injection of neutralizing antibodies to control irradiated mice did not significantly affect the cumulative percentage of survival. Therefore, endogenously produced IL-1, IL-6, TNF, and SCF induced by AS101 appear to contribute to the survival of irradiated mice pretreated with this agent.

Effect on spleen cell and BM cellularity. In the following set of experiments, mice were irradiated with sublethal doses of irradiation (450 cGy). At this dose of irradiation, AS101 has previously been shown to protect various cellular functions at different time points after irradiation.[17] For the purpose of studying the effect of neutralizing antibodies against various cytokines on the radioprotective effect of AS101, day 5 after irradiation was selected. Figures 2A and B show that, after subjection to a sublethal dose of irradiation, there was evidence of an earlier recovery of spleen and BM cellularity in mice injected with AS101 before irradiation. Five days after 450 cGy of irradiation, the number of spleen cells in PBS-injected mice was less than 10% of normal, and the number of BM cells was 14% of normal nonirradiated mice. These values increased to nearly normal levels in mice pretreated with AS101. AS101 (10 μg/mouse) increased the number of BM cells from 4.1 ± 0.8 to $27.5 \pm 3.1 \times 10^6$/2 femurs ($P < .01$) and increased the number of spleen cells from 7.5 ± 0.6 to $125 \pm 7.5 \times 10^6$/spleen ($P < .01$). Treatment of mice with anti-IL-1R antibodies nearly abrogated the increase in spleen and BM cellularity in AS101-treated mice. The number of BM and spleen cells in AS101-injected and irradiated mice was not significantly different from that of nonirradiated mice (Fig 2). Similar results were obtained when mice were treated with anti-IL-6R, anti-TNF, or anti-SCF antibodies. However, no abrogation of the increase in BM and spleen cellularity in AS101-treated mice was observed when anti-IL-2R antibodies or the control relevant Igs were used.

Effect on cytokines secretion. Preliminary studies in our laboratory showed that the secretion of GM-CSF, IL-6, and

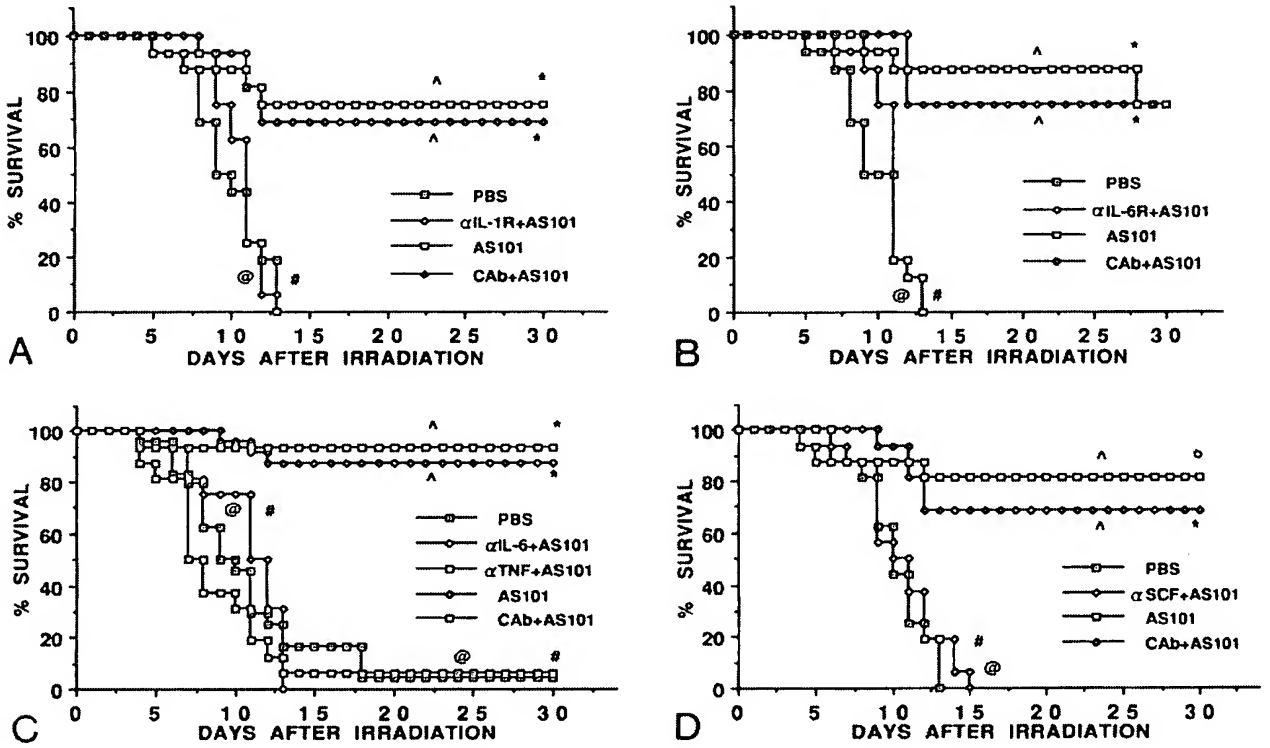

Fig 1. Effect of anticytokine antibodies on the radioprotection by AS101. BALB/c mice received 100 μg of anti-IL-1R antibody intraperitoneally, (A); 100 μg anti-IL-6R (Ab), (B); 100 μg anti-TNF Ab and 600 μg anti-IL-6 Ab, (C); and 100 μg anti-SCF Ab, (D). Control mice received the same amounts of relevant Igs. Mice were treated with 0.1 mL of the neutralizing antibodies followed immediately by intraperitoneal injection of 10 μg AS101. Twenty-four hours later, injected mice were irradiated with 750 cGy. The rate of survival of mice injected with AS101 and 100 μg anti-IL-2R Ab was 90% 30 days after irradiation. Results represent a total of 16 mice per group. *, $P < .01$ increase versus PBS by Gehan-Wilcoxon test; ^, $P < .01$ increase versus PBS by χ^2 test; #, $P < .01$ decrease versus AS101 by Gehan-Wilcoxon test; @, $P < .01$ decrease versus AS101 by χ^2 test.

IL-3 by spleen cells of irradiated mice is significantly decreased as early as 24 hours after irradiation (data not shown). Pretreatment with AS101 24 hours before sublethal irradiation restores the ability of spleen cells to secrete, in the presence of ConA, all three cytokines as examined at various time points after irradiation.

Five days after irradiation, a significant ($P < .01$) decrease in the secretion of several cytokines including GM-CSF, IL-6, and IL-3 was observed. (Figs 3 through 5). Treatment with AS101 increased the level of IL-3 and IL-6 to levels that were indistinguishable from those observed in nonirradiated mice. The level of GM-CSF was also significantly increased. Pretreatment of mice with each of the anticytokine antibodies (except anti-IL-2R antibody) significantly decreased IL-3, GM-CSF, and IL-6 levels in AS101-treated animals AS101 ($P < .01$).

Role of specific cytokines in the hematopoietic recovery conferred by AS101. To study the role of cytokines in AS101-induced hematopoietic recovery, mice were sublethally irradiated and injected with AS101 in combination with one of the various neutralizing antibodies. Although mice irradiated with 450 cGy survived for 7 months, there was a marked effect on peripheral blood hematology. Five days after irradiation the number of white blood cells decreased from 5.4 ± 0.44 to 0.59 ± 0.03 × $10^3/\mu L$. The number of platelets decreased from 805 ± 28 to 538.5 ± 24.5 × $10^3/\mu L$ and that of neutrophils decreased from 1.09 ± 0.14 to 0.1 ± 0.01 × $10^3/\mu L$. The number of lymphocytes was also significantly decreased. As can be seen in Table 1, the hematopoietic recovery in AS101-treated mice was complete 5 days after irradiation. This recovery was totally abrogated when any of the four antibodies (IL-1R, IL-6R, TNF, and SCF) were injected to mice treated with AS101 ($P < .01$ for all antibodies). In contrast, control Igs did not significantly affect the AS101-induced hematopoietic recovery (data not shown).

DISCUSSION

The data presented in this study clearly show a significant role for specific cytokines in the radioprotective effect of the immunomodulator AS101. The results indicate that AS101-induced endogenous production of four cytokines (IL-1, TNF, IL-6, and SCF) is required for hematopoietic recovery and for the survival of mice subjected to sublethal or lethal irradiation.

We have recently shown that radioprotection conferred by

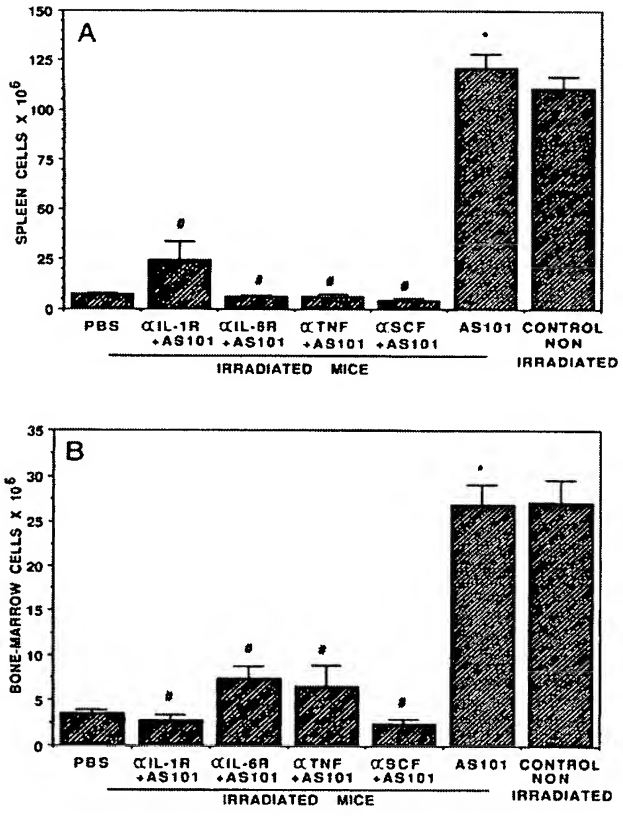

Fig 2. Effect of anticytokine antibodies on the recovery of spleen and BM cellularity induced by AS101 in sublethally irradiated mice. Mice were killed 5 days after 450 cGy irradiation. Spleens and femurs were removed, and single-cell suspensions were prepared and counted. (A) recovery of spleen cells; (B) recovery of BM cells. The number of spleen cells in mice injected with AS101 and anti-IL-2R Ab was 129.3 ± 24.7 × 10^6; with AS101 and control IgG_1 was 110.48 ± 14.1 × 10^6; with AS101 and control IgG_2, 107.5 ± 11.62 × 10^6. The number of BM cells in mice injected with AS101 and anti-IL-2R Ab was 24.3 ± 2.1 × 10^6; with AS101 and control IgG_1, 26.36 ± 3.4 × 10^6; and with AS101 and control IgG_2, 18.6 ± 1.4 × 10^6. Results represent mean ± SE of 18 mice per group. (PBS; AS101; control nonirradiated) and 6 mice per group (neutralizing antibodies). *, $P < .01$ increase versus PBS; #, $P < .01$ decrease versus AS101.

AS101 is associated with induction of progenitor cells to enter into S phase,[17,21] which is known to be the most radioresistant phase of the cell cycle.[25] Also, AS101 enhanced the stimulation of spleen colony-forming units (CFU-S) toward proliferation and CFU-S self-renewal.[21] Moreover, we showed that the DNA repair processes expressing the cellular responses associated with the restoration of the normal nucleotide sequence after damage caused to the DNA were also significantly increased after treatment with AS101.[26] Early progenitor cells have been previously reported to be relatively more resistant to various DNA-damaging agents.[27,28] This resistance is likely to result from a more efficient repair mechanism. Therefore, it is quite possible that AS101 accounts for the faster rate of DNA repair by the increased number of stem cells in the tested population of cells.

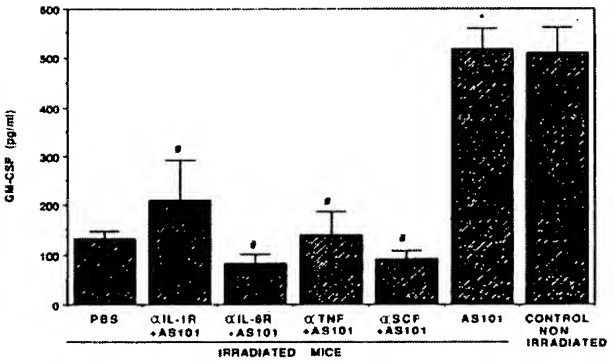

Fig 3. GM-CSF secretion by spleen cells of irradiated mice pretreated with AS101 combined with anticytokine antibodies. Mice were killed 5 days after 450 cGy irradiation. Culture of spleen cells stimulated with ConA were prepared. GM-CSF was quantified in supernatants. Levels of GM-CSF in mice treated with AS101 and anti-IL-2R Ab were 369.5 ± 65.5 pg/mL; with AS101 and control IgG_1, 426.8 ± 26.6 pg/mL; and with AS101 and control IgG_2, 629.4 ± 128.6 pg/mL. All these values were not statistically different from AS101 and $P < 0.01$ versus PBS. Results represent mean ± SE of 18 mice per group (PBS, AS101, control nonirradiated) and 6 mice per group (neutralizing antibodies). *, $P < .01$ increase versus PBS; #, $P < .01$ decrease versus AS101.

Injection of AS101 into mice has been previously shown to increase simultaneously the endogenous secretion of a variety of cytokines such as IL-1, IL-6, IL-2, and GM-CSF.[12-17,24] Phase I clinical trials on cancer patients treated with AS101 showed enhanced production of TNFα, γ-IFN, and IL-2.[18,19] Preliminary results in our laboratory show increased production of SCF by human BM stromal cells incubated with

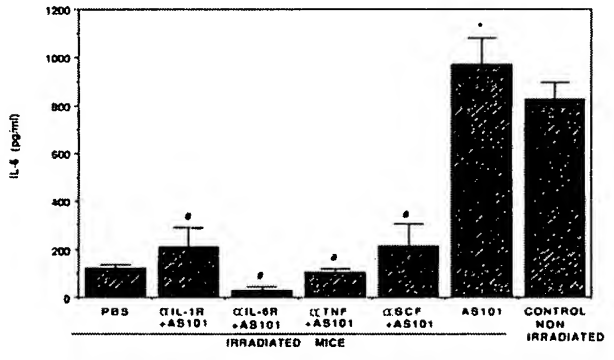

Fig 4. IL-6 secretion by spleen cells of irradiated mice pretreated with AS101 combined with anticytokine antibodies. IL-6 was analyzed in supernatants of spleen cells removed from mice 5 days after irradiation. IL-6 levels in mice treated with AS101 + anti-IL-2R Ab were 1,012 ± 157 pg/mL; with AS101 plus control IgG_1, 827.7 ± 112.9 pg/mL; and with AS101 plus control IgG_2, 1,121 ± 245.7 pg/mL. All values were not statistically different from AS101 and $P < .01$ versus PBS. Results represent mean ± SE of 18 mice per group (PBS, AS101, control nonirradiated) and 6 mice per group (neutralizing antibodies). *, $P < .01$ increase versus PBS; #, $P < .01$ decrease versus AS101.

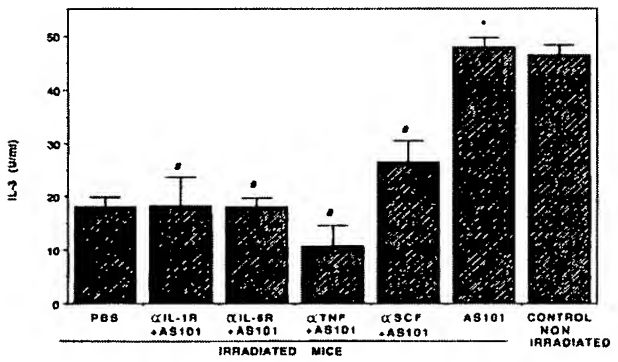

Fig 5. Effect of anticytokine antibodies on IL-3 production by spleen cells of irradiated mice pretreated with AS101. IL-3 was prepared from spleen cells of irradiated mice killed 5 days after irradiation. Levels of IL-3 in mice treated with AS101 plus anti–IL-2R Ab were 40.65 ± 3.1 U/mL; with AS101 plus control IgG_1, 42.8 ± 3.4 U/mL; and with AS101 plus control IgG_2, 52.8 ± 4.2 U/mL. All values were not statistically different from AS101 and $P > .01$ versus PBS. Results represent mean ± SE of 18 mice per group (PBS, AS101, control nonirradiated) and 6 mice per group (neutralizing antibodies). *, $P < .01$ increase versus PBS; #, $P < .01$ decrease versus AS101.

AS101 in vitro (Kalechman et al, unpublished results). Some of the above-mentioned cytokines have been previously reported to protect mice if injected, either alone or in combination, before irradiation. For example, IL-1 has been reported to radioprotect mice when administered 20 hours before irradiation.[7] IL-1 and TNFα have been reported to have an additive effect in radioprotection,[8] and the c-*kit* ligand has been recently reported to protect mice from lethal effects of radiation.[11] The injection of IL-6 by itself did not improve radiation survival; however, IL-6 has been shown to act synergistically with suboptimal doses of IL-1 to enhance survival from lethal irradiation.[29]

Because AS101 was able to simultaneously induce the production of a variety of radioprotective cytokines, it was hypothesized that this agent might protect mice from the toxic effect of irradiation by enhancing secretion of these factors.

The availability of neutralizing antibodies to cytokines or their receptors allowed the assessment of the contribution of the individual cytokines to radioprotection by AS101. Neutralizing antibodies were injected simultaneously with AS101, because our aim was to treat mice with these antibodies 16 to 20 hours before the increase in endogenous cytokine production induced by AS101 occurred.

It is of interest to note that, although AS101 stimulates simultaneously a variety of cytokines, injection of one of neutralizing antibodies to IL-1R, IL-6R, TNF-α, or SCF completely reduces the protective effect of AS101. These results suggest that radioprotection by AS101 can be conferred only with increased secretion of all four cytokines. However, these may induce yet another unknown factor that confers the radioprotection.

The interdependence and synergistic interactions of these four cytokines is well documented. Several studies suggested that many of the actions of IL-1 or TNF, including stimulation of production of acute-phase proteins by hepatocytes,[30,31] stimulation of the hypothalmic-pituitary adrenal axis,[32] or hematopoietin-1[33] effects, can be mimicked by IL-6.[34,35] In addition, SCF has been reported to act as a potent comitogen for hematopoietic stem cells in combination with IL-6, IL-3, and IL-1.[36] The absolute requirement of a variety of cytokines for hematopoietic recovery from lethal irradiation has been previously reported. The administration of IL-6 antibody blocked IL-1– and TNF-induced radioprotection.[37] In addition, antibodies to IL-1R abrogated TNF-induced radioprotection.[38] Recently, the neutralization of SCF has been shown to reduce the survival of irradiated mice treated with the radioprotectants lipopolysaccharide and IL-1.[39]

The observation that cytokines are more effective in combination rather than when administered separately lends further support to the notion that these agents act in concert and that, despite their apparent redundency, they all must be present for normal host defense. The ability of AS101 to induce the secretion of a variety of cytokines suggests that it is able to accelerate the restoration of functional hemopoietic cells, because the administration of each of these factors stimulates a broader spectrum of progenitor cells than anticipated. For example, high doses of GM-CSF stimulated multipotential (CFU–granulocyte-erythroid-macrophage-megakaryocyte) and erythroid (CFU-E and burst-forming unit E) progenitor cells in addition to the expected myelogenous cells.[40] Thus, interaction of the different mediators may be required for increased hematopoiesis. Moreover, the induction of endogenous cytokines is more effective than their exogenous administration. Vogel et al[41] have reported that

Table 1. Peripheral Blood Hematology of Mice 5 Days After 450 cGy Irradiation

	Nonirradiated Control	Irradiated					
		PBS	AS101*	AS101 + αIL-6R†	AS101 + αTNFα†	AS101 + αSCF†	AS101 + αIL-1R†
WBCs × $10^3/\mu L$	5.4 ± 0.44	0.59 ± 0.03	6.0 ± 0.46	0.77 ± 0.24	0.73 ± 0.02	0.89 ± 0.31	0.66 ± 0.07
Plts × $10^3/\mu L$	805.6 ± 28.2	538.5 ± 24.5	897.1 ± 30.4	501 ± 40.9	616 ± 25.16	411.4 ± 37.3	505.6 ± 26
Neutrophils × $10^3/\mu L$	1.09 ± 0.14	0.1 ± 0.01	1.02 ± 0.12	0.21 ± 0.03	0.2 ± 0.06	0.02 ± 0.01	0.11 ± 0.02
Lymphocytes × $10^3/\mu L$	4.34 ± 0.32	0.3 ± 0.03	4.05 ± 0.29	0.32 ± 0.9	0.19 ± 0.09	0.17 ± 0.13	0.21 ± 0.05

Results represent the mean ± SE of 18 mice per group (PBS, AS101, control nonirradiated) and 6 mice per group (neutralizing antibodies).
Abbreviations: WBCs, white blood cells; Plts, platelets.
* $P < .01$ increase versus PBS.
† $P < .01$ decrease versus AS101.

after intraperitoneal administration of a single dose of rIL-1, high titers (2 to 3 × 10^7 U/mL) of CSF appeared in the circulation within 2 hours and persisted for up to 6 hours. This level is higher than CSF concentration achieved after intraperitoneal administration of 20,000 U of GM-CSF.

It is important to note that the elevation in cytokine production increased threefold to sixfold in irradiated mice as compared with irradiated antibody-treated mice, despite the equal number of spleen cells in the ConA-stimulated cultures of both groups. This is probably because of the protection of T-cell functions rather than the protection of mature $CD4^+$ cell numbers. This explanation rests on the previously reported capability of AS101 to protect the ability of spleen cells of irradiated mice, in which no decrease in the proportion of $CD4^+$ cells occurred, to proliferate and secrete IL-2.[20]

In summary, the present study shows that endogenous production of IL-1, IL-6, TNF, and *kit* ligand in mice injected with AS101 provides a major contribution to radioprotection by this agent. The data suggest that the simultaneous production of all four cytokines by AS101 is a prerequisite for its protection from radiation lethality. The possibility that these relatively toxic cytokines can be replaced by the nontoxic immunomodulator presents an obvious clinical advantage.

A better understanding of the mechanism by which AS101 confers radioprotection will enable us to use AS101 more effectively for the restoration of hemopoiesis in patients after radiation therapy or in those suffering from overdose or accidental irradiation.

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